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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
HUTSON, RICHARD G	
ART UNIT	**PAPER NUMBER**
1652	

DATE MAILED: 03/10/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/607,903	HUISMAN ET AL.	
	Examiner	Art Unit	
	Richard G. Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23* is/are pending in the application.

4a) Of the above claim(s) *11-23* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/2003*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicants preliminary amendment the specification and claims 1, 2, 4, 9, 11, 13 and 18, in the paper of 6/27/2003, is acknowledged. Claims 1-23 are still at issue and are present for examination.

Election/Restrictions

Applicant's election with traverse of Group I, Claims 1-10 in the paper of 12/14/2005, is acknowledged. Applicants have correctly repeated the relationship between the elected group I and the non-elected group II, as being drawn to a product and a process of use of the product, respectively. As pointed out by applicants upon the determination of an allowable product claim, those claims drawn to the methods of use of that product, wherein said method claims require all of the limitations of the allowable product claim will be rejoined.

The requirement is still deemed proper and is therefore made FINAL.

Claims 11-23 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper."

Applicants filing of information disclosure statement filed on 6/27/2003, is acknowledged. Those references considered have been initialed.

Claim Objections

Claims 1, 9, 10 are objected to because of the following informalities:

Claims 1 recites "...in less that 24 hours". It is suggested that this be amended to "...in less than 24 hours".

Claim 9 (claim 10 dependent on) recites "... the bacterial strain of claim 1 wherein the nuclease is encoded..." It is noted that claim 1 does not refer to a "nuclease" but rather a "nuclease gene" and a "nuclease gene product". The nuclease reference in claim 9 is interpreted as being the same as the "nuclease gene product" in claim 1. It is suggested that applicants clarify such.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-10 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-10 are drawn to any bacterial strain for the production of a fermentation product, wherein the bacteria strain is genetically modified to express a heterologous nuclease gene or mutated to improve the activity of a homologous or heterologous nuclease gene, wherein the nuclease gene product is secreted into the periplasm or growth medium resulting in an amount effective to degrade at least 95% of all nucleic acid released following lysis of the cells in less than 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery is enhanced. (claims 1, 4, 5, and 10), wherein the bacterial strain is capable of growth to cell densities of at least 50 g/l (claim 2) or produces a polyhydroxyalkanoate to a level of 40% of its dry cell weight (claim 3) wherein the nuclease gene is a heterologous or a homologous gene (claims 6, 9), wherein the host strain is selected from the group consisting of *Ralstonia eutropha*,...*Escherichia coli* and *Klebsiella* (claim 7), wherein the nuclease is expressed in an amount effective to degrade at least 95 % of all nucleic acid released by lysis of the cells in less than 24 hours (claim 8).

The specification fails to describe representative species of these bacterial strains by any identifying structural characteristics or properties other than the functional characteristics recited in the claims, for which no predictability of structure is apparent. There is no disclosure of any particular structure to function/activity relationship in the disclosed species with respect to those heterologous nuclease genes or those genetic modifications of homologous nuclease genes such that expression or modification is in an amount effective to degrade nucleic acid so that recovery of a product is enhanced.

The genus of bacterial strains that are claimed is a large variable genus comprising any bacterial strain comprising any heterologous nuclease gene as well as any genetic modification of any homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. The specification discloses only the species of the claimed genus encompassed by *P. putida*, *R.. eutropha* and *E. coli*, expressing the heterologous *Staphylococcus aureus* nuclease gene, *nuc*, which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. Applicants do not describe a single bacterial strain which comprises a genetic modification of a homologous nuclease gene such that expression or modification is an amount effective to degrade nucleic acid so that recovery of a product is enhanced. Therefore, one skilled in the art cannot reasonably conclude that the Applicant had possession of the claimed invention at the time the instant application was filed.

Appellant is referred to the revised interim guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 4, 5, 6 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Liebl et al. (J. Bacteriology 174(6): 1854-1861 (1992)).

Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Coryn*ebacterium glutamicum* is closely related to other "amino acid-producing corynebacteria" and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Therefore, Liebl et al. anticipates claims 1, 2, 4, 5 and 8 drawn to a bacterial strain wherein the bacterial strain expresses a heterologous nuclease gene. It is noted that with respect to claims 4 and 5, that Liebl et al. do not teach that this bacterial strain is used in a process to manufacture poly(3-hydroxyalkonates) or polysaccharides, but since these are uses of the recited bacterial strain these are not patentable limitations of the claimed bacterial strain, thus these claims are included in the rejection. While Liebl et al. do not teach the limitations of claims 2 and 8 with respect to density of growth and amount of enzyme produced, respectively, it is believed that these are inherent properties of the disclosed bacterial strain absent some teaching to contrary. As shown in Figure 2, Liebl et al. show that one of the taught bacterial strains, *C. glutamicum* R163/pWLQN10, produced approximately 80-fold higher SNase activity after induction as a result of IPTG supplementation of the medium and this strain grew approximate

density of OD_{600} = 5.0 (Figure 2 and supporting text at page1859, right column, last paragraph before Discussion). Compare this optical density to that of the instantly disclosed strains MBX978 and MBX 985 which Appellants disclose as having an OD_{600} of 3.7 and 3.8 at the end of disclosed experiments (Table 1 of instant application).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 1-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

Greer et al. teach that the degradation or removal of nucleic acids from cell lysates during fermentation is important because they form solutions of high viscosity which interfere with subsequent processing. Greer et al. specifically teach the usefulness of peroxide degradation in the recovery of intracellularly produced materials,

in particular polyhydroxyalkanoate polymers, from bacterial cell lysates. Greer et al. further teach that nucleases can also be added to a cell lysate in order to degrade the nucleic acid although nucleases are expensive (page 1, lines 25-31).

Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Corynebacterium glutamicum is closely related to other "amino acid-producing corynebacteria" and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Miller et al. teach the secretion and processing of *Staphylococcal aureus* nuclease in *Bacillus subtilis*.

Atkinson et al. teach all aspects of biochemical engineering and biotechnology, including properties of microorganisms, microbial activity, product formation, fermentation processes, downstream processes and product recovery processes. Atkinson et al. also teach many products that can be produced biochemically such as antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides. Atkinson et al. specifically teach many of the industrial production characteristics for a number of commercially important compounds, for example Atkinson et al. teach that Alcaligenes eutrophus has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of

approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32).

Lee et al. teach several processes developed for the production of various poly(hydroxyalkanoic acids) including various microorganisms used and the optimization of fermentation conditions.

One of ordinary skill in the art would have been motivated to genetically engineer a bacterial strain to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process for the synthesis of industrially important molecules. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would a inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and

Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous nuclease to increase its nuclease activity for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

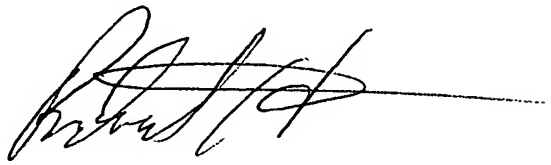
Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, consisting of antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Methylobacterium organophilum*, *Methylobacterium extorquens*, *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans*, *Pseudomonas acidovorans* and *Escherichia coli* or any other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that Alcaligenes eutrophus has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain

which grows to a high cell density and/or which produces a high level of the desired product.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy can be reached on 571-272-0928. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



Richard G Hutson, Ph.D.
Primary Examiner
Art Unit 1652

Rgh
3/1/2006